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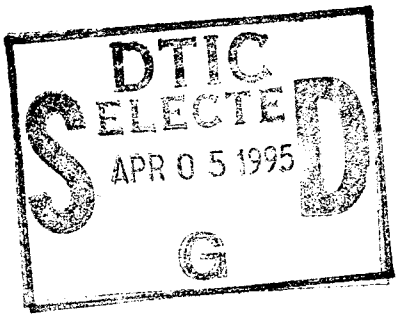
Waterways Experiment Station

Dredging Operations Technical Support Program

Protocol for Conducting Sediment Bioassays with Materials Potentially Containing Zebra Mussels (*Dreissena polymorpha*)

by Jerre G. Sims, David W. Moore, WES
Elayne Gamble, AScI Corporation



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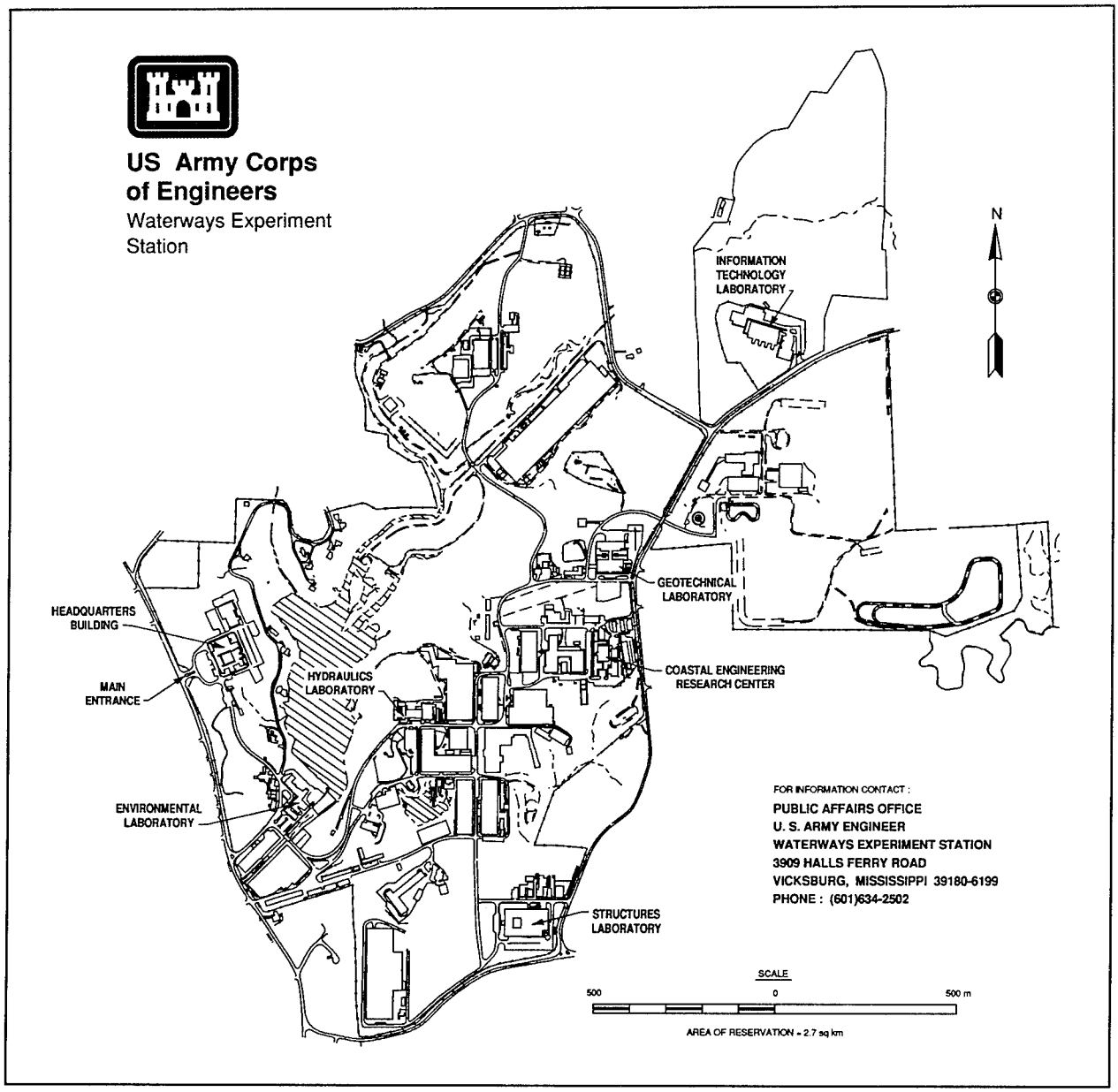

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Environmental Effects of Dredging Programs

Dredging Operations Technical Support

Report Summary

Protocol for Conducting Sediment Bioassays with Materials Potentially Containing Zebra Mussels (Dreissena polymorpha) (MP D-95-1)

ISSUE: Concerns have been expressed over the potential for accidental release of zebra mussels from sediment testing facilities. Since the accidental release of the zebra mussel in the United States in 1985, many waters have become infested, and distribution of the organism has become widespread. However, procedures are being developed to avoid release in waters that are not infested and to avoid additional release in waters already infested. A protocol for receiving, handling, and testing sediment and water potentially containing *D. polymorpha* was developed to guide the conduct of sediment bioassays.

RESEARCH: An investigation was made of the rationale and requirements of Public Law 101-646 (the Nonindigenous Aquatic Nuisance Prevention and Control Act). Physical characteristics of the organism *D. polymorpha*, its current and potential distribution, and treatment and control methods were also examined.

SUMMARY: Guidance is provided concerning facility design, personnel requirements and training, procedures during sample handling and testing, and treatment of equipment and materials prior to removal from a sediment testing facility.

AVAILABILITY OF REPORT: The report is available on Interlibrary Loan Service from the U.S. Army Engineer Waterways Experiment Station (WES) Library, 3909 Halls Ferry Road, Vicksburg, MS 39180-6199; telephone (601) 634-2355.

To purchase a copy, call the National Technical Information Service (NTIS) at (703) 487-4780. For help in identifying a title for sale, call (703) 487-4780. NTIS report numbers may also be requested from the WES librarians.

About the Authors: Ms. Jerry Sims is a biologist in the WES Environmental Laboratory (EL), and Dr. David Moore is a research biologist, EL. Ms. Elayne Gamble is a research assistant with AScI Corporation. For further information about the Dredging Operations Technical Support Program, contact Mr. Thomas R. Patin, Program Manager, at (601) 634-3444.

Contents

Preface

The work reported herein was conducted by the U.S. Army Engineer Waterways Experiment Station (WES) for the U.S. Army Engineer Division, North Central. The point of contact was Mr. Jan Miller (Operations). Financial support was provided by the Dredging Operations Technical Support (DOTS) Program, Mr. Thomas R. Patin, Manager. The DOTS Program is managed through the Environmental Effects of Dredging Programs, Dr. Robert M. Engler, Manager.

This report was prepared by Ms. Jerre G. Sims of the Environmental Processes and Effects Division (EPED), Environmental Laboratory (EL), WES, Ms. Elayne Gamble of the AScI Corporation, and Dr. David W. Moore of EPED, EL, WES. It was based upon a protocol developed for the Aquatic Biological Effects Team by Ms. Gamble.

Technical input was provided by Mr. Jeff Denny, U.S. Environmental Protection Agency, Duluth, MN, Dr. Dan J. Hornbach, Macalester College, St. Paul, MN, Dr. W. Gregory Cope, National Fisheries Research Center, La Crosse, WI, Drs. Andrew C. Miller and Barry S. Payne, WES, Dr. Gerry Nichols, Fish and Wildlife Service, Ann Arbor, MI, and Dr. Dave Reed, Great Lakes Environmental Research Laboratory, Ann Arbor, MI.

Technical review was provided by Drs. Andrew Miller, Barry Payne, and Edwin A. Theriot of the Environmental Resources Division, EL, and Dr. Tom Dillon of the EPED, EL.

Permission to use the copyrighted material from *Monitoring and Control of Dreissena polymorpha and Other Macrofouling Bivalves in The Netherlands* and *Controlling Zebra Mussel (Dreissena polymorpha) Veligers with Three Oxidizing Chemicals: Chlorine, Permanganate, and Peroxide + Iron* was obtained from CRC Press, Inc./Lewis Publishers.

The work was performed under the general supervision of Dr. Bobby L. Folsom, Jr., Chief, Fate and Effects Branch, EPED. The Chief of EPED was Mr. Donald L. Robey, and the Director of EL was Dr. John W. Keeley.

At the time of publication of this report, Director of WES was Dr. Robert W. Whalin. Commander was COL Bruce K. Howard, EN.

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1 Introduction

The report herein describes a protocol for receiving, handling, and testing sediment and/or water potentially containing *Dreissena polymorpha* (zebra mussel). It was prepared as a result of concerns over the potential for accidental release of *D. polymorpha* from sediment testing facilities. Included is a brief discussion of the distribution and potential distribution of *D. polymorpha*. Guidance includes facility design, personnel requirements, procedures used during sample handling and testing, and treatment of equipment and materials prior to removal from a sediment testing facility.

2 Background

Public Law

The Nonindigenous Aquatic Nuisance Prevention and Control Act (Public Law 101-646) includes provisions to develop a protocol to ensure that research carried out under Subtitle C of the Act does not result in the unintentional release of aquatic nuisance species into navigable waters of the United States (Reid et al. 1993). The rationale for establishing such protocol is based on the economic, ecological, or disruptive impacts caused by nuisance organisms, along with previous experience that research has sometimes been solely responsible for introduction of nonindigenous species into waters of the United States. The Act requires (a) the prevention of unintentional introduction and dispersal of non-indigenous species into waters of the United States through ballast water management and other requirements; (b) the coordination of federally conducted, funded, or authorized research, prevention, control, information dissemination and other activities regarding the zebra mussel and other aquatic nuisance species; (c) the development and implementation of environmentally sound control methods to prevent, monitor, and control unintentional introductions of nonindigenous species from pathways other than ballast water exchange; (d) the understanding to minimize economic and ecological impacts of nonindigenous aquatic nuisance species that become established, including zebra mussels; and (e) the establishment of a research program and technology development to assist states in the management and removal of *D. polymorpha*.

The Organism

Dreissena polymorpha is a macrofouler that quickly colonizes in new areas and rapidly achieves high densities. It attaches to hard substratum such as rocks, logs, aquatic plants, shells of native mussels, and exoskeleton of crayfish. It can also attach to plastic, concrete, wood, fiberglass, pipes made of iron and polyvinyl chloride, and surfaces covered with conventional paints (Miller, Payne, and McMahon 1992).

Dreissena polymorpha is a freshwater bivalve mollusc, native to Europe. The common name, zebra mussel, is derived from dark brown or green bands that alternate in a "zebra like" striped pattern with white to cream-colored background bands. This color pattern, shape, size, and presence of numerous byssal threads distinguish it from other bivalves native to United States waters. *Dreissena polymorpha* has many physical and biological characteristics different from native bivalves (Mackie 1991). It attaches itself to solid substrate via the byssal threads. Females can produce 30,000 to 1,000,000 eggs annually. Planktonic veligers, which are zebra mussel larvae, are microscopic and can be carried by water currents. Adults and juveniles can attach to hulls, motors, boat trailers, etc., to facilitate transport. Adults can reach sizes up to 2 to 3 cm.

Distribution

The initial colonization resulted from a European cargo ship releasing its ballast water to Lake St. Clair in 1985 (Herbert, Muncaster, and Mackie 1989; Carlton and Geller 1993). By 1989, zebra mussels were found in water intake pipes in industrial and municipal water plants in Lakes St. Clair, Erie, and Ontario; and the distribution was found to have expanded from the Great Lakes to the Illinois River. Additionally, in Monroe, Michigan, densities as high as 700,000 zebra mussels/square meter were reported. By 1990, zebra mussels were found in all the Great Lakes. By 1991, *D. polymorpha* had been collected in major river systems throughout the Eastern United States.

The potential distribution of *D. polymorpha* includes much of North America. Serious infestations are reported to likely occur in inland navigational systems (Miller, Payne, and McMahon 1992). Shallow, warm lake bottoms are a primary target of *D. polymorpha*, with large hard-water lakes and running waters more than 30 m wide also included (Strayer 1991).

Control

All materials coming into contact with sediment or water from regions of the country known or suspected to be infested with *D. polymorpha* must be treated prior to disposal or removal from a confined test facility. There are a number of effective treatment procedures for the destruction of *D. polymorpha*. Two of the most common methods are heat and chemical oxidants. Because of the health hazards associated with the use of chemical oxidants and the high concentrations and holding times required for destruction of adult zebra mussels, heat treatment is preferred (Jenner and Janssen-Mommen 1993). Where practical, treatment procedures described in this protocol rely on heat. A temperature of 36 °C has been found to result in 100-percent mortality of adult *D. polymorpha* after less than 30 min of exposure (Jenner and Janssen-Mommen 1993, Figure 1).

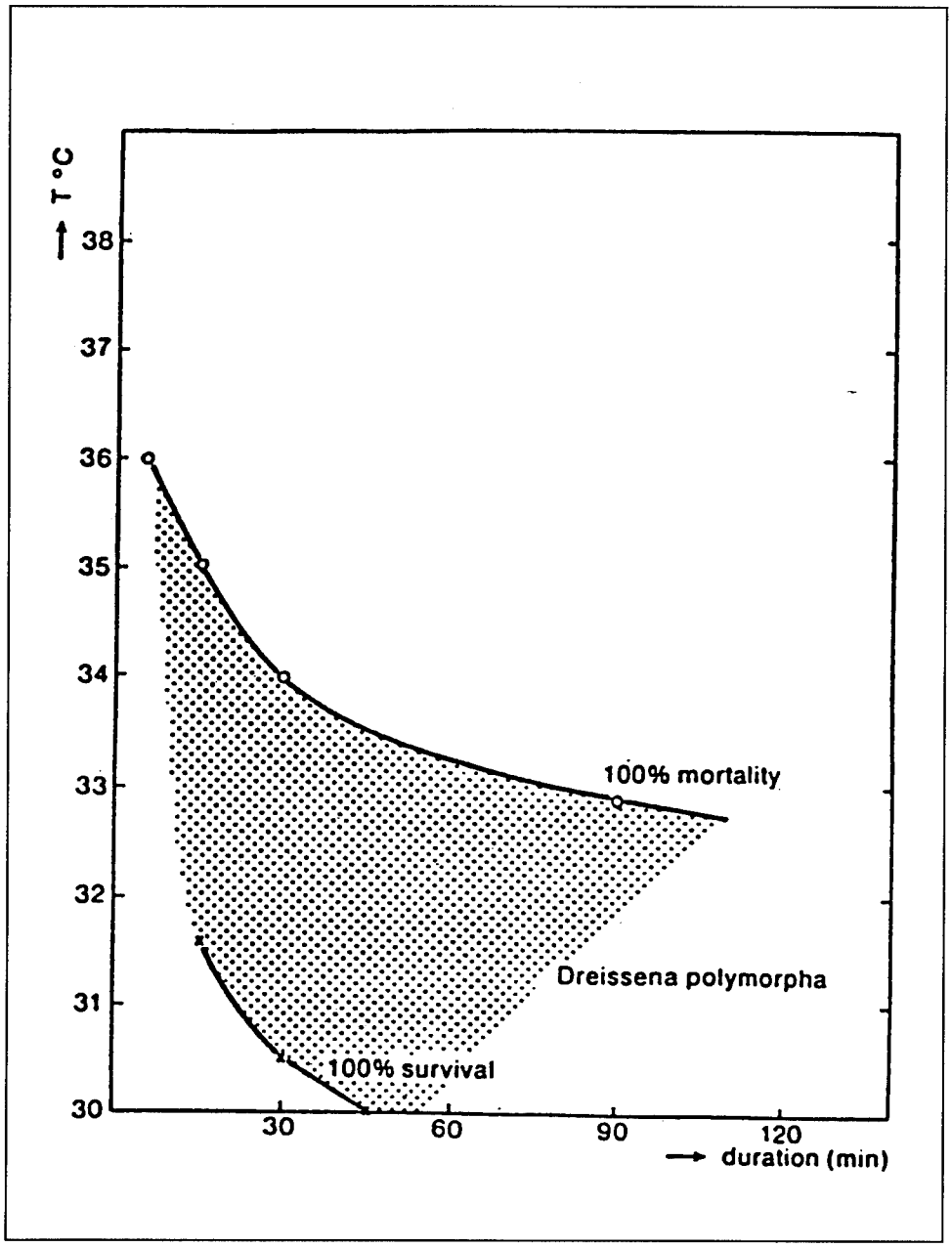

Figure 1. Survival and mortality of adult *D. polymorpha* exposed to a range of temperatures (30 to 36 °C) (from Jenner and Janssen-Mommen 1993)

Some circumstances (e.g., larger pieces of equipment and instrumentation) may require the use of a chemical oxidant such as a dilute chlorox solution todestroy veligers. Klerks, Fraleigh, and Stevenson (1993) has shown concentrations of greater than or equal to 0.5 mg/L result in 100-percent mortality of *D. polymorpha* veligers within 2 hr (Figure 2). Techniques other than those described herein may be used, but the effectiveness (i.e., 100-percent destruction of adults and veligers) of these techniques must be documented.

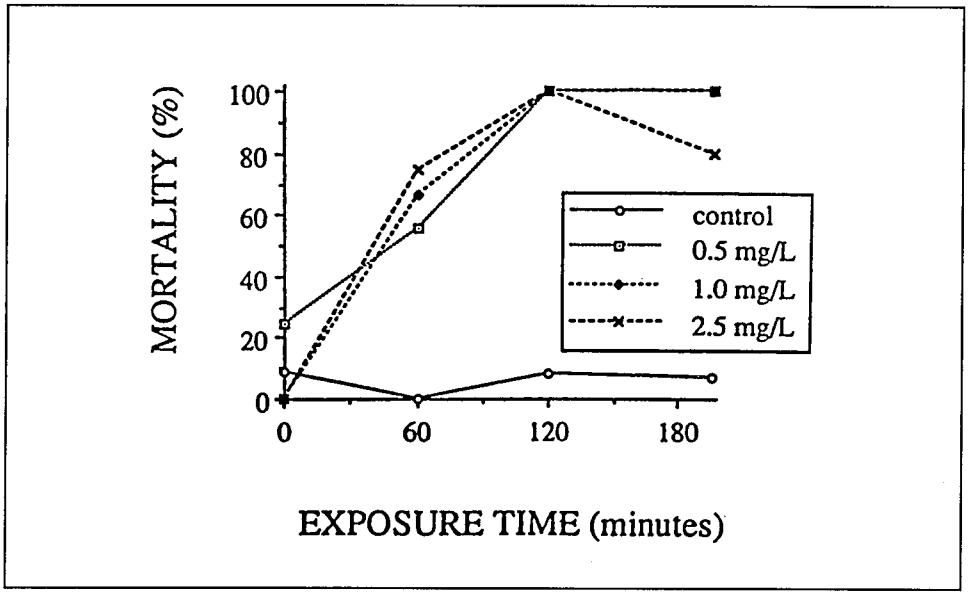

Figure 2. Mortality of *D. polymorpha* veligers exposed to applied chlorine concentrations of 0.5, 1.0, and 2.5 mg/L (from Klerks, Fraleigh, and Stevenson 1993)

3 Protocol

Applicability

This protocol applies to all dredged material bioassays involving material from water bodies known or suspected to be infested with *D. polymorpha*.

Containment Room Design

A containment room should be designed for receiving, handling, and testing sediment and/or waters potentially containing *D. polymorpha*. The containment room should have all floor and sink drains capped and sealed to prevent unintentional release of organisms. Entrance to the containment room should be locked at all times with keyed access only by authorized personnel. Authorized personnel are those individuals who have been assigned to the project and/or have received proper training and are familiar with the protocol. A sign-in/sign-out sheet should be posted just inside the door. Signs should be posted on the entrance/exit doors of the containment room and near other points of potential release (i.e., sink drains, vents, windows, etc.) Signs may include the following:

Entrance Side of Door:
ENTRY BY AUTHORIZED PERSONNEL ONLY
Testing of Sediments and/or Water Samples
Containing Zebra Mussels
For Additional Information Contact
(Authorizing Persons Name)

Exit Side of Doors:
Do Not Remove Any Equipment, Apparel, and/or Glassware From This
Room Without Prior Treatment and/or Authorization
For Further Information Contact
(Authorizing Persons Name)

Above Sinks:
DRAINS HAVE BEEN SEALED
All Water Must Be Collected
And Treated Prior To Disposal

Personnel Requirements

Only authorized personnel are to enter the containment room. Personnel entering the containment room should be required to sign in and put on appropriate apparel, if necessary (i.e., disposable laboratory coats, gloves, rubber boots, and protective eyewear). All protective apparel should remain in the containment room in the proper storage area. When exiting the containment room, personnel should be required to remove disposable laboratory coats and gloves and place them in autoclavable waste bags. Waste bags should be autoclaved prior to disposal. Nondisposable protective apparel such as rubber boots and glasses should be removed and wiped with a 0.10-percent chlorox solution, rinsed with water, thoroughly dried, and then returned to the designated storage area. A 1,000-fold dilution (0.10 percent) of household bleach (5.25 percent NaOCl by weight) equals to approximately 102 mg Cl_2/L, which is well above concentrations known to be effective for control of zebra mussel veligers (Klerks, Fraleigh, and Stevenson 1993, Figure 2). Personnel should sign out prior to exiting the containment room.

Sample Handling Procedures

Receipt of sediments and/or waters contaminated with *D. polymorpha*

All arriving samples should be placed in the containment room prior to opening and sample log-in. All samples should be entered into a sample log book. Log book entries should include the following information:

a. Project name.

b. Names of persons receiving/checking samples.

c. Date and time of arrival at the laboratory.

d. Chain-of-custody sheet (i.e., whether or not a chain-of-custody sheet was sent, if so, who released samples, their affiliation, and reference location of where chain-of-custody sheets will be kept).

e. Origin of shipment (i.e., when and where samples were obtained, sample identification, collection site, and number of samples).

f. Temperature of sample.

g. Type of sample containers (e.g., 5-gal (19-L) plastic pail and 55-gal (200-L) steel drum).

h. Verbatim record of the labeling of sediment containers and, if appropriate, laboratory designation or codification of samples.

i. Method of shipment (e.g., Federal Express and UPS) and appropriate reference number or document number (e.g., airbill number).

j. Condition of containers (see Emergency Procedures - Containers damaged during shipment).

k. Sample description (i.e., general appearance, odor, presence of adult *D. polymorpha*, and amount of material, etc).

Sieving

If sieving is required, then all sediments should be sieved in the containment room. Prior to disposal, materials retained on a sieve should be treated according to procedures described for sediment and organisms under Treatment of Material, Supplies, and Equipment Prior to Removal from Containment Room. Once sediment has been sieved, the sieve should be treated according to procedures outlined for small equipment.

The exterior of sealed pails or other containers used to hold the sieved sediments should be rinsed with water and the rinse water collected and treated according to procedures outlined for wastewater under water section. The exterior of sealed pails should then be sponge wiped with a 0.10-percent chlorox solution prior to removal from the containment room for cold storage.

Containers used to confine sediment and collected rinse water during sieving should be treated according to procedures outlined for small equipment.

Mixing/homogenization

All mixing and homogenizing of sediments should be done in a containment room. A large electric mixer may be used to mix the sediment. Once sediment has been homogenized, the mixer should be removed from the sediment and the mixer blades removed and rinsed with water. Rinse wastewater should be collected in the holding vessel and treated according to procedures outlined for wastewater under Treatment of Material, Supplies, and Equipment Prior to Removal from Containment Room. Blades should be treated according to procedures outlined for small equipment.

The exterior of sealed pails or other containers used to hold homogenized sediment should be rinsed with water and the rinse water collected and treated according to procedures outlined for wastewater. The exterior of sealed pails should then be sponged wiped with 0.10-percent chlorox solution prior to removal from the container room for cold storage.

The container used to confine sediment and collected rinse water during mixing/homogenization should be treated according to the procedure outlined for large equipment.

Conducting Sediment Bioassays with Sediments and/or Water Samples Containing *D. polymorpha*

All testing should be conducted solely in the containment room. No glassware or equipment should be removed from this room without treatment and/or authorization.

Treatment of Material, Supplies, and Equipment Prior to Removal from Containment Room

Water

All wastewater should be collected and heated to 50 °C for 2 hr in a holding vessel (55-gal (200-L) steel drum with an immersion heater is suitable for this purpose). A continuous recording thermometer should be used to ensure that a temperature of 50 °C is maintained for 2 hr. Following heat treatment, wastewater should be removed from the containment room and disposed of in an appropriate waste treatment facility.

Sediment

All test sediments should be collected in heat resistant pails. Sealed pails should be placed in a hot water bath (50 °C) for 2 hr prior to disposal (the holding vessel described above is also suitable for this purpose). A continuous recording thermometer should be used to monitor sediment core temperature and ensure that a temperature of 50 °C has been maintained for a 2 hr period.

Organisms

Any adult *D. polymorpha* initially removed from sediment via sieving should be heat treated as described above for sediments.

Disposable supplies

All disposable supplies should be collected in autoclavable bags and autoclaved prior to disposal.

Small equipment

Any nondisposable equipment that is appropriate to fit inside the hot water bath described above (e.g., beakers, scoops, sieves, and mixer blades) should be heat treated for 2 hr prior to cleaning and according to the laboratory's normal standard operating procedures (SOPs).

Large equipment

Any nondisposable equipment too large to fit inside the hot water bath described above (e.g., aquaria, water baths, and containment vessel) should be rinsed with water to remove adhering sediment and other material. Rinse water should be collected and treated as described above. Following this initial rinse, the equipment should then be wiped down with a 0.10-percent chlorox solution prior to cleaning and cleaned according to normal laboratory SOPs.

Emergency Procedures

Containers damaged during shipment

When sediments and/or water samples arrive, all containers should immediately be taken to the containment room and inspected. If there is a broken seal, the container should be resealed, if possible, unless sediment mixing is imminent. The exterior of the container should be wiped with a 0.10-percent chlorox solution. If the container is damaged and/or cannot be resealed, the material should be transferred to an undamaged container. Prior to disposal, the damaged container should then be treated according to procedures described for small equipment under Treatment of Material, Supplies, and Equipment Prior to Removal from Containment Room. In the event of damaged or leaking containers, the project officer (the individual given responsibility for the project) should be notified.

Accidental spills

A pre-designated mop and bucket should be used to collect spills. Following collection of spilled material, the spill area should be damp mopped with a 0.10-percent chlorox solution. Collected waste should be subjected to treatment procedure outlined above (Treatment of Material, Supplies, and

Equipment Prior to Removal from Containment Room - Water). In the event of a spill, the project officer should be notified.

Natural disaster threats

If there is a threat of a natural disaster, such as a tornado, flood, or hurricane, special precautions to ensure safe confinement of test material should be considered.

Personnel to be notified

A prioritized list of appropriate personnel to contact (including both office and home phone numbers) in the event of an emergency should be located in the containment room.

Training

All laboratory personnel should become familiar with this protocol. They should be trained in the appropriate SOPs. A waterproof copy of this protocol should be posted in the containment room and other appropriate locations.

4 Summary

The protocol described herein was developed to ensure that no accidental release of *D. polymorpha* occurs during the conduct of sediment bioassays. This is a comprehensive containment and treatment plan that will ensure accomplishment of this goal.

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13. ABSTRACT *(Maximum 200 words)*

The zebra mussel, *Dreissena polymorpha*, was introduced in United States waters accidentally in 1985 and has since become a major nuisance organism. Although many waters have become infested and distribution of the organism has become widespread, procedures are being developed to avoid release in waters that are not infested and to avoid additional release in those that are already infested.

Techniques and procedures to avoid the unintentional release of the nonindigenous species during conduct of bioassays are discussed. This protocol contains methods for receiving and handling sediment and/or water samples used in bioassays that may potentially contain zebra mussels. Facility design, personnel requirements, effective treatments, and emergency guidelines are presented in the report.

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